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ORAL ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Sustained MYB Activity Is Necessary for Oncogenic Transcription in *KMT2A*-Rearranged Acute Lymphoblastic Leukemia through Enhancer-Promoter Interactions and Epigenetic Modifications at Enhancers

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Aberrant enhancer activity is a hallmark of many cancers including *KMT2A*-rearranged acute lymphoblastic leukemia (ALL), an aggressive leukemia subtype with an event-free survival of 19-45% in infant-ALL (<1 year of age) and 50-59% in childhood-ALL cases. Targeting enhancer activity may provide a novel therapeutic strategy for this hard-to-treat disease. Transcription factors are key effectors of enhancer activity. They recognise and bind to specific DNA sequences at enhancers, driving the expression of target genes from these distal loci. MYB is an important hematopoietic transcription factor that is commonly overexpressed in *KMT2A*-rearranged leukemias and is required for leukemia survival, where loss of MYB has been shown to abrogate the leukemia phenotype. However, the precise mechanism by which MYB contributes to enhancer function, and the stages of enhancer activation at which it acts are unknown.

We employed a well-characterized chromatin anchoring system to interrogate MYB function and determine whether it can recruit specific enhancer activities *de novo*. By fusing the MYB transactivation domain (MYB ^{TA}) to the Tet repressor (TetR) DNA binding domain, MYB ^{TA} is directed to an array of Tet operator (*TetO*) sequences incorporated into a neutral genomic region devoid of native gene regulatory elements. We found that MYB ^{TA} alone is sufficient to recruit key enhancer-associated proteins, including P300, Brd4 and Mediator to the *TetO* locus. We observed MYB ^{TA}-dependent deposition of H3K27ac, increased chromatin accessibility and transcription not just at the *TetO* locus, but remarkably, also at regions more than 50kb away. Using the chromosome conformation capture (3C) technique NG Capture-C, we observed MYB ^{TA}-induced DNA interactions between *TetO* and the distal sites of transcription, as well as more long-range interactions up to 400kb distal to where MYB ^{TA} is bound. Together, these observations suggest that MYB ^{TA} binding alone is sufficient to establish an enhancer-like regulatory element, which activates distant cryptic promoters. This activity is dependent on the continued presence of MYB ^{TA}, as disruption of MYB ^{TA} binding results in a rapid loss of transcription and acquired chromatin features, implicating MYB in enhancer maintenance as well as initiation.

To confirm our observations in a disease context, we tested the requirement for MYB at endogenous enhancers in SEM cells, a model of *KMT2A*-rearranged ALL. By stably introducing a degradation tag (FKBP12 ^{F36V}) to the endogenous *MYB* gene, we were able to rapidly induce protein degradation and observe the immediate effects on chromatin features. Using transient transcriptome sequencing, we noted significant transcriptional downregulation of known MYB target genes, including *BCL2*, *MYC*, *LMO4* and *CDK6*. We also observed decreases in enhancer H3K27ac levels and eRNA transcription, most marked at MYB-bound loci. To investigate the requirement for MYB in maintaining physical proximity between enhancer and promoter, we performed the base pair-resolution 3C technique Micro-Capture-C (MCC). MCC revealed highly punctate interactions between enhancer elements and their target promoters. At *BCL2* and *MYC*, MYB degradation results in a reduction in interactions with the promoter at discrete regions within the enhancers. This argues for highly localised MYB-dependent activity at enhancers, driving interaction with and activation of distal target promoters.

Overall, our data argue for a role for MYB in establishing enhancers *de novo*, indicating that MYB overexpression may directly result in onco-enhancer activity at key genes *in vivo*. At a subset of MYB-bound enhancers, the continued presence of MYB is absolutely required to maintain enhancer activity and oncogene upregulation. Further exploration of associated co-factors that mediate MYB-dependent enhancer activity may identify novel targets for therapeutic exploitation.

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